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Ingested Mineral
Fibers: Elimination in
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Ingested Mineral Fibers: Elimination in Human Urine

Abstract. *Sediment in human urine examined by transmission electron microscopy contains amphibole fibers which originate from the ingestion of drinking water contaminated with these mineral fibers. The ingestion of filtered water results in the eventual disappearance of amphibole fibers from urine. These observations provide the first direct evidence for the passage of mineral fibers through the human gastrointestinal mucosa under normal conditions of the alimentary canal.*

A key question in the evaluation of the possible human cancer risk associated with the ingestion of asbestos and other durable fibers in water, beverages, and food is whether microscopic fibers under normal conditions of the alimentary canal can migrate through the gastrointestinal mucosa (1). Such movement of fibers could lead to their incorporation into the bowel wall or, after hematogenous or lymphatic transport, into the peritoneum and other organ tissues. Occupational exposure to airborne asbestos dust appears to be associated with increased incidence of gastrointestinal and peritoneal cancer as well as asbestosis, bronchiogenic carcinoma, and pleural mesothe-

lioma (2). Inhalation of asbestos dust is accompanied by the ingestion of many fibers cleared from the respiratory tract by mucociliary action (3).

The introduction of large concentrations of asbestos and other durable fibers into the pleura (4), peritoneum (5), or respiratory tract (6) of rodents results in malignant neoplasms. Rats fed asbestos fibers for long periods have failed to provide evidence of tumor production (7), but rats fed powdered chrysotile asbestos filter material (47 percent nonasbestos material included) had a significant excess incidence of malignant tumors over controls (8).

Some studies of tissues from animals

Table 1. Amphibole fiber concentrations in urine samples.

Subject*	Sex	Age (years)	Exposure†	Amphibole fiber concentration‡ (fibers per milliliter) in	
				Urine	Blank sample
A	Male	82	UF	310 (194-469)	8 (0.2-30)
G	Male	27	UF	590 (370-893)	<14 (0-52)
I	Female	55	UF	580 (289-1038)	90 (19-263)
J	Female	46	UF	1170 (562-2152)	<70 (0-258)
B	Male	48	UF + W	150 (41-384)	<20 (0-74)
C	Female	48	UF + F	10 (0.2-56)	<5 (0-18)
H	Female	53	UF + F	<30 (0-111)	<10 (0-37)
K	Male	46	UF + F + W	120 (77-179)	10 (1-36)
G'	Male	27	F	40 (13-93)	<10 (0-37)
L	Male	37	F	40 (5-144)	<15 (0-55)
A'	Male	83	F	13 (0.3-72)	5 (0.1-28)
D	Female	39	F + W	20 (3-72)	<7 (0-26)
E	Male	36	F + W	<20 (0-74)	<7 (0-26)
F	Female	30	F + W	20 (0.5-111)	<11 (0-41)
M	Male	25	N	<20 (0-74)	<6 (0-22)
N	Male	40	N	<30 (0-111)	<10 (0-37)

*Volunteer subjects are alphabetically listed in chronological order of sample collection and analysis. G' and A' designate second samples from subjects G and A collected approximately 2 months and 1 year, respectively, after ingestion of filtered tap water began in January 1977. †Codes for ingestion exposure (22) to amphibole fibers in drinking water during the month preceding sample collection: UF = unfiltered Lake Superior water (high amphibole fiber concentration); F = filtered Lake Superior water (low amphibole fiber concentration); W = well water (amphibole fiber concentration below detection limits); and N = never ingested Lake Superior water. ‡Measured amphibole fiber concentrations or detection limits are reported as fibers per milliliter of urine with a 95 percent confidence interval for a Poisson distribution indicated within parentheses. Blank sample concentrations or detection limits are reported as calculated for the corresponding urine sample volume.

that had ingested fibers provide no evidence of fiber transport through the gastrointestinal mucosal lining (7), but evidence for such movement is reported in other studies (9). Scanning electron micrographs showing large amosite asbestos fibers penetrating epithelial cells of rat jejunal mucosa tissue were recently reported (10). Mice that drank water suspensions of latex spheres 2 μ m in diameter for 2 months were found to have the latex particles accumulated in macrophages in intestinal Peyer's patches (11). Latex particles 0.22 μ m in diameter were reported to migrate from rat stomachs to lymphatics of the mucosa and also to liver and kidney tissues (12). Much larger particles of silica, opal phytoliths from plants, have been observed in digested mesenteric lymph node and kidney tissue from sheep which eat cereal chaff and grains (13).

Evidence for the uptake in the human intestine of particles as large as 75 μ m is provided by the observation of starch granules in blood only minutes after ingestion (14). Measurements made while subjects were sleeping, smoking, or consuming caffeinated beverages detected higher quantities of starch particles in the blood. Dyed cellulose particles have also been identified in human blood and urine after the ingestion of specially stained plant foods (15). The cellulose fibers are not found in urine until at least 1 week after ingestion. Direct evidence of the movement of asbestos and other inorganic fibers in the human body after

their ingestion has not been reported.

No excess incidence of gastrointestinal cancer mortality (16) or morbidity (17) has been detected among residents of Duluth, Minnesota, after nearly 18 years of ingesting unfiltered Lake Superior water contaminated with amphibole fibers (18). We recently carried out detailed transmission electron microscope examinations of sediment filtered from urine samples collected from Minnesota residents with varying exposures to drinking water drawn from western Lake Superior. We report here the identification of amphibole fibers and other inorganic particles in the urine of persons who drank unfiltered Lake Superior water.

Urine specimens (50 to 500 ml) were voided into particle-free glass flasks without flask-skin contact. The flask was immediately capped with aluminum foil to prevent contamination by airborne dust. All subsequent sample manipulations were conducted in a filtered-air environment. A filtered distilled water sample (19) accompanied each urine sample as a blank through the analytical procedures.

Each urine and blank sample was slowly vacuum-filtered through either a cellulose acetate membrane filter (diameter, 47 mm; mean pore diameter, 0.45 μ m) or a polycarbonate membrane filter (diameter, 47 mm; mean pore diameter, 0.2 μ m). Precipitation of organic material during storage interfered with microscope analysis, and so each sample was

filtered as soon as possible after collection. The membrane filters were ashed at low temperature; the ash was resuspended in filtered distilled water and acidified to approximately pH 4 to prevent precipitation of phosphates and other salts, and the resulting suspension was filtered through a polycarbonate membrane filter (diameter, 25 mm; mean pore diameter, 0.1 μ m). Drinking water samples were filtered through polycarbonate membrane filters (diameter, 47 mm; mean pore diameter, 0.1 μ m). Electron microscope grids were prepared from carbon-coated membrane filters with a technique described for water samples (20).

In the transmission electron microscope examination of samples we systematically scanned (at a magnification of $\times 10,000$ or higher) locator grid openings of approximately 9500 μ m². Each particle with a length-to-width ratio equal to or greater than 3:1 was measured and recorded. Amphibole, chrysotile, and other mineral fibers were identified by morphology and selected-area electron diffraction. Energy-dispersive x-ray fluorescence spectra obtained with the transmission electron microscope were used to determine the elemental compositions of the individual fibers. Fiber concentrations were calculated from the number of fibers identified in examined grid openings which represented a known sample volume of urine (21).

Urine samples were obtained from individuals who were categorized into four different exposure groups as determined by the type of drinking water ingested (22) in the month prior to sample collection (Table 1). All subjects, except two, had some known prior exposure to drinking water and food contaminated with amphibole fibers. All subjects were in good health, and none reported any history of kidney disease or occupational exposure to asbestos fibers. Analyses of air samples from the Duluth area indicate very low or nondetectable concentrations of amphibole fibers.

The concentrations of amphibole fibers in urine (Table 1) are reported with 95 percent confidence intervals based on Poisson distributions of fibers demonstrated by a variance test (23) for the fiber count data. Only for individuals who drank unfiltered water did the urine samples contain more than ten times the number of fibers corresponding to the detection limits (10 to 40 amphibole fibers per milliliter of urine). Blank samples contained either no detectable amphibole fibers or concentrations much less than those measured in the corresponding urine samples. Thus, amphi-

bole fibers observed in urine are not the result of contamination introduced during sample preparation or analysis.

The amphibole fiber concentrations (Table 1) show a clear association with the type of drinking water. The urine of subjects A and G, after months of ingesting only filtered water, contained less than 1/10 the amphibole fiber concentrations found in the urine when these subjects drank only unfiltered water. A further test of drinking water as a source of amphibole fibers in urine is provided by a comparison of energy-dispersive x-ray spectra obtained from amphibole fibers identified in urine and drinking water. All fibers analyzed contained silicon, iron, and magnesium. Many also contained calcium, and some gave detectable manganese K α peaks. For almost all the fibers, sodium, aluminum, and potassium were not detected. The magnesium-iron-calcium ternary diagrams (Fig. 1) demonstrate the almost identical distributions of fiber chemistries in the urine and drinking water samples. Comparison with mineral standards indicates an amphibole mixture of approximately 75 percent cummingtonite-grunerite [(Fe,Mg)₇Si₈O₂₂(OH)₂], 20 percent actinolite [Ca₂(Fe,Mg)₅Si₈O₂₂(OH)₂], and 5 percent other amphibole minerals. Few tremolite or anthophyllite fibers, which often were present in commercial tales (24), were identified.

The mean amphibole fiber length and width for urine samples from subjects who drank unfiltered Duluth water are 0.96 and 0.17 μ m, respectively. This mean width is little different from the mean width (0.20 μ m) measured for amphibole fibers in several 1976 Duluth water samples, but these water samples had a larger mean amphibole fiber length (1.42 μ m). The previously reported (20) mean amphibole fiber length of 1.98 μ m and width of 0.30 μ m for Duluth water samples reflect larger fiber sizes caused by storm resuspension of settled amphibole particles in Lake Superior. The data suggest that the transport of ingested fibers from the gastrointestinal tract to urine may occur preferentially for shorter fibers, although amphibole fibers up to 10 μ m long were observed in urine.

Other mineral particles are commonly identified in urine samples. In addition to nonfibrous particles of silica, diatom fragments, iron, and clay minerals, fibers of iron, titanate, and glass of probable man-made origin are observed. Chrysotile fibers are abundant in most urine samples but are also present in blank samples (25). Chrysotile, fibrous glass, and other inorganic fibers are used in filters for beverage, food, and parenteral

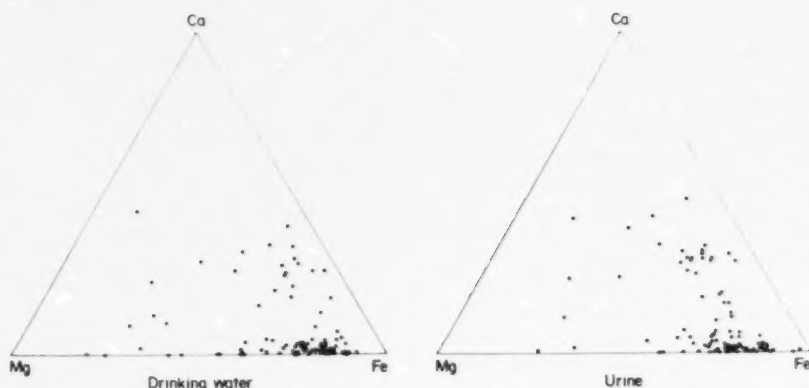


Fig. 1. Iron-magnesium-calcium ternary diagrams for the major cation compositions of 100 amphibole fibers in both unfiltered drinking water and urine from persons drinking the water. Plotted cation x-ray emission intensity ratios are calculated directly from integrated K α peak intensities obtained after background subtraction from the energy-dispersive x-ray fluorescence spectra of individual amphibole fibers.

drug preparation and can contaminate the filtrate (26).

A 1953 report (27) of the identification by optical microscopy of asbestos fibers 4 to 6 μ m long in the urine of asbestos workers proposed that the inhaled fibers had been transported through the alveolar wall to the circulatory system. The fraction of inhaled fibers available for such transport is quite small since most are exhaled, captured in the nasal passages, or rapidly cleared from the lung by mucociliary action to the gastrointestinal tract (3). Thus it would be necessary for an individual to inhale very high concentrations of chrysotile fibers in order to produce, solely by migration from the lung to the urinary tract, the concentrations we measured in urine. The amphibole fibers seen in the urine samples can be attributed only to ingestion.

Passage of micrometer-sized fibers through the kidney is not consistent with the organ's ability to retain much smaller protein material. The ultrafiltration process across the glomerular capillary wall retains protein molecules as small as an effective molecular radius of 40 Å (28). Small amounts of protein that pass the glomerular filter are reabsorbed in the proximal tubule, but reabsorption is not expected to affect inorganic particles which reach the tubule. Exercise, stress, or extremes of heat or cold can influence glomerular ultrafiltration.

The accumulation of fibers in kidney tissue is a potential cause of renal disease. An excess of deaths attributable to kidney carcinoma (20 observed versus 8 expected) was recently reported for a large cohort of asbestos insulation workers (29). Male rats examined histopathologically after drinking for 3 months water containing chrysotile fibers were reported to have foci of damage in the

kidney cortex and to have red blood cells and hyaline casts in the urine sediment (30). Four kidney carcinomas were observed among 12 malignant tumors found for a group of 50 rats fed ground asbestos filter material (8). Two malignant tumors (not in the kidney) occurred among 50 control rats. Chronic interstitial nephritis as a result of the ingestion of quartz and silicate particles has been attributed to damage caused by silicic acid rather than by particles transported to the kidneys (31).

Concentrations of amphibole fibers eliminated in urine (Table 1) represent approximately 10^{-3} of the number of fibers ingested with drinking water. This ratio seems remarkably large and may be modified by further measurements. Diurnal variations in kidney function, for example, were not accounted for since only one 24-hour specimen (subject A) was analyzed. The magnitude for intersubject variability in gastrointestinal mucosa, kidney, and other tissue permeability to particles is also unknown.

Many complex pathways with different time factors undoubtedly exist for particle transport from the gastrointestinal tract to urine. Despite these complications, an approximate steady state between the number of ingested fibers and the number of fibers eliminated in urine could exist so that the number of fibers in urine would represent the minimum number of fibers absorbed by the intestinal mucosa. To the extent that some fibers are permanently retained by the body or eliminated by other routes, the urine concentrations are an underestimate of the ingested fiber absorption.

The time needed to reach such a steady state, or to reach zero urine elimination of fibers after the cessation of ingestion, should equal the time necessary

for all fibers from a single exposure to be eliminated or permanently retained in tissue. A single day's ingestion of water contaminated with amphibole fibers was observed to result in a urine amphibole fiber concentration approximately 10^{-3} of the number of fibers ingested, with fibers detectable in urine at least 10 days after ingestion. Subject G, after drinking filtered water for 2 months, had a 90 percent reduction in the urine amphibole fiber concentration. Subject A, 13 months after a change to filtered water, had a greater reduction in the urine amphibole fiber concentration.

The data suggest that some ingested mineral fibers are accumulated in body tissue but do not allow a prediction of whether fibers are permanently retained. Further study of ingestion exposure and urine clearance should allow calculation of steady-state body concentrations of those fibers subject to eventual elimination in urine. Furthermore, the electron microscope analysis of urine samples for inorganic particles such as asbestos fibers can provide a valuable index of past exposure.

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18. P. M. Cook, G. E. Glass, J. H. Tucker, *Science* **185**, 853 (1974). Amphibole minerals are members of a class of hydrated silicates having a double-chain crystal structure. These minerals can crystallize during rock formation in an asbestiform habit (bundles of individual fibers). Asbestiform amphibole minerals used commercially as asbestos are amosite (fibrous grunerite), crocidolite, anthophyllite, tremolite, and actinolite. Most amphibole particles in western Lake Superior water fall in the cummingtonite-grunerite series. A fiber in a water sample is defined as any particle with a length-to-width ratio equal to or exceeding 3:1. Some nonasbestiform amphibole mineral crystals, when crushed, form cleavage fragments that are microscopically very similar, or identical, in morphology, crystal structure, and chemistry to small fibers which result from the crushing of amphibole asbestos. Approximately 95 percent of the asbestos used in North America is chrysotile, a fibrous serpentine mineral with a distinct tubular microstructure.
19. Distilled water, filtered successively through membrane filters with mean pore diameters of 0.45 and 0.1 μ m, was used for all glassware cleaning, sample preparation, and blank sample analysis.
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21. Analysis of a sample with a measured addition of amphibole fibers indicated a 29 percent underestimate of amphibole fibers, due probably to interference of urine sediment and failure to completely disperse clumps of ashed sample. Similar measurements on air samples containing amphibole fibers ashed at low temperatures have produced equivalent results. These measures of accuracy only relate to sample loss caused by low-temperature ashing and refiltration. Inability to see and identify all fibers is a problem common to the electron microscope analysis of any particle sample [D. Beaman and D. File, *Anal. Chem.* **48**, 101 (1976); (15)].
22. Unfiltered Duluth drinking water ingested by subjects A, B, and G is estimated by combined x-ray diffraction and electron microscope analysis (20) to have contained approximately 50×10^6 amphibole fibers per liter, significantly less, as a result of seasonal effects on Lake Superior water circulation, than the annual average concentration of 120×10^6 amphibole fibers per liter. Subjects I and J drank unfiltered water from both the Duluth and Two Harbors, Minnesota, water supplies and probably had the greatest daily intake of amphibole fibers. Subject K had very little exposure to unfiltered water except for the ingestion of Duluth water with 8×10^6 amphibole fibers per liter 2 days before the urine sample reported in Table I was collected. Filtered drinking water generally contained less than 1 percent of the amphibole fiber concentration of unfiltered drinking water. The entire Duluth municipal water supply has been filtered since January 1977.
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25. The primary source of blank sample chrysotile fiber contamination is the membrane filters which are ashed at low temperature along with the sample. Careful analysis of different lots of filters makes it possible to select filters with minimum chrysotile contamination. Chrysotile fiber concentrations in urine, based on five samples analyzed with low chrysotile contamination filters, may often exceed 1000 fibers per milliliter. A further complication in the evaluation of the measured chrysotile fiber concentration in urine is the possible breakdown of ingested chrysotile fiber bundles into many very small unit fibrils either during fiber migration through the body or during sample preparation procedures. We find that chrysotile fibrils subjected to simulated stomach acidity conditions (stirred for 3 hours in filtered distilled water with pH 1.8 at 38°C) retained their structural integrity and gave diagnostic electron diffraction patterns. Some magnesium is probably leached as observed in fibers isolated from lung tissue [F. Pooley, *Br. J. Ind. Med.* **29**, 146 (1972)] or in dissolution studies [J. Thomassin, J. Goni, P. Baillif, J. Touray, M. Jaurand, *Phys. Chem. Minerals* **1**, 385 (1977)].
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